PARASITISM OF MYXOMYCETE PLASMODIA ON FUNGOUS MYCELIA

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With plate 54

TO FURTHER the general thesis that the Myxomycetes play a rôle in the consumption of fungi which cause wood decay, the digestion and assimilation of the mycelia of chiefly lignicolous fungi by plasmodia was studied after the parasitism of the plasmodial stage of several Myxomycetes upon mushrooms and polypores had been demonstrated (5). Enlightenment upon the problem was sought by observation of the habits of plasmodia in the forest and also by laboratory tests of the feeding habits of plasmodia on pure cultures of numerous fungi. This study has brought to light the mycophagous habit in several species of Myxomycetes, in addition to those already reported (5), and has disclosed a wide range of fungous mycelia capable of being digested by plasmodia.

RELEVANT LITERATURE

The digestion of the mycelia of fungi by slime molds has, with but one or two exceptions, been reported only incidentally by investigators in connection with their study of some other phase of the biology of the group. The work of A. Lister (6) gives us some excellent notes on the behavior of the plasmodium of *Badhamia utricularis*, but only the reaction of the plasmodium to the mycelium of a chance, unidentified fungus was observed. Hilton (3) cultivated *B. utricularis* upon moist bread and he observed that the hyphae of various species of *Aspergillus* and *Penicillium*, which developed on the bread, were dissolved and absorbed by the plasmodium.

Physarum nutans has been credited by Elliott and Elliott (2) with the absorption and destruction of the mycelia of *Bulgaria polymorpha* and *Coryne sarcoides* within the wood of an oak branch. Sanderson (7) frequently encountered *Physarum auriscalpium* Cooke upon the mycelium of *Sphaeronema fimbriatum* which rots tapped surfaces of *Hevea; Physarum reniforme* Lister on the dead cortex of *Hevea* attacked by *Ustulina zonata;* and *Arcyria denudata* Wettst. associated with *Sphaerostilbe repens* in Malaya on fuel heaps of split timber and on small diseased roots of *Hevea*. One cannot read Sanderson's paper without feeling that the plasmodia of Myxomycetes may parasitise fungi responsible for the decay of wood.

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Skupienski (9) describes the plasmodium of *Didymium difforme* feeding on *Aspergillus glaucus*, *Sterigmatocystis* sp., *Penicillium* sp., *Stysanus* sp., and various other molds, yeasts, and bacteria. He also found that the plasmodium of *Didymium nigripes* would digest the hyphae of *Penicillium* (8). On the other hand, Celakovsky (1) working with *Chondrioderma* (*Didymium*) *difforme* Rost. reported plasmodia as non-mycophagous, since he observed plasmodia envelop spores and hyphae of *Penicillium glaucum*, *Mucor stolonifer* and *Phycomyces nitens*, and later relinquish them unharmed.

MATERIALS AND METHODS

The plasmodia used in these studies were collected in the forest and brought into the laboratory for cultivation on media, or were cultivated directly from spores (4). The cultures of fungi employed were either isolated by the authors or obtained through the generosity of other workers, especially Dr. Irene Mounce and Dr. C. L. Shear.

In the earlier trials (Tables 1 and 2), in order to test the parasitism of plasmodia, petri plates of nutrient agar were inoculated in the center with the fungus being tested, which was allowed to grow until the colony covered about one-half the diameter of the dish before a bit of plasmodium, previously freed from contaminating fungi, was transferred to the culture. The various standard nutrient agar media used for the culture of the fungi included rolled oat, ground corn, ground corn and dextrose, malt extract, and potato dextrose.

In later trials (Table 3), the senior author devised the following technique: using a sterilized instrument, a disk about one centimeter in diameter is cut from the center of a layer of two per cent plain agar in a petri plate (Plate 54, fig. 1) and in the vacancy is inserted a similar disk of nutrient agar upon which the test fungus is growing. When the fungous hyphae grow from the nutrient agar disk into the plain agar, a piece of plasmodium is introduced and its action toward the hyphae observed. This change in technique was made because in previous experiments where a nutrient substratum was employed and where the plasmodium very slowly digested the mycelium, it was difficult to ascertain whether the growth of the myxomycete was due to digestion of the fungus or to absorption of nutrients directly from the agar. In spite of repeated attempts, it was impossible to free certain of the plasmodia from bacterial contaminants, so here again, the non-nutrient, slightly acid agar substratum was an advantage, as it helped to inhibit the growth of bacteria. In the later trials the H-ion concentration of the agars was taken into account and was adjusted to pH. 6.0 ± 0.3.

THE PARASITIC HABIT AND THE INFLUENCING FACTORS

In order first, to determine if the digestion of fungous hyphae by Myxomycetes is affected by the nutrients in different culture media, as has been reported for the consumption of some bacteria by plasmodia, and second, to find a favorable medium, the junior author made a few preliminary experiments in which fungi were grown on five different agar media, namely, potato dextrose, linseed, *Vicia*

TABLE 1.—PARASITISM OF PLASMODIA UPON FUNGI GROWING ON DIFFERENT MEDIA.

Fungus / Myxomycete	Badhamia magna	Badhamia rubiginosa	Badhamia utricularis	Brefeldia maxima	Hemitrichia clavata	Leocarpus fragilis	Lindbladia effusa	Lycogala epidendrum	Physarum cinereum	Physarum flavicomum	Physarum polycephalum	Physarum virescens	Trichia decipiens
CORN DEXTROSE AGAR													
1. Collybia velutipes	VP	—	—	P	P	—	VP	P	P	—	VP	P	P
2. Pleurotus ostreatus	VP	P	—	P	P	—	—	—	P	—	—	—	—
3. " serotinus	VP	P	—	P	P	—	—	—	P	—	—	—	—
4. Polyporus resinosus	P	N	—	N	P	—	—	—	P	—	—	—	—
ROLLED OAT AGAR													
1. Pleurotus serotinus	VP	P	—	—	—	—	—	—	VP	—	—	—	—
2. Polyporus resinosus	—	P	—	—	—	—	—	—	—	—	—	—	—
POTATO DEXTROSE AGAR													
1. Fomes applanatus	—	P	—	—	P	—	—	—	—	—	—	—	—
2. Pleurotus ostreatus	—	N	VP	N	N	—	—	—	—	VP	VP	—	—
3. " serotinus	—	N	VP	—	—	—	—	—	—	VP	VP	—	—
4. Polyporus resinosus	P	N	—	—	—	—	—	—	—	P	P	—	—
LINSEED AGAR													
1. Collybia velutipes	—	N	—	—	N	—	—	—	—	—	—	—	—
2. Fomes applanatus	—	N	—	N	—	—	—	—	—	—	—	—	—
3. Pleurotus ostreatus	—	P	—	N	—	VP	—	—	—	—	—	—	—
VICIA FABA AGAR													
1. Fomes applanatus	—	—	—	—	—	—	—	—	VP	—	—	—	—
2. " fomentarius	N	—	—	—	—	—	—	—	—	—	—	—	—
3. " pinicola	—	—	—	—	—	—	—	—	N	—	—	—	—
4. Polyporus resinosus	N	—	—	—	—	—	—	—	—	—	—	—	—
5. Polystictus versicolor	VP	—	—	—	—	—	—	—	—	—	—	—	—

N—not parasitic, P—parasitic, VP—very parasitic.

Faba, rolled oat, and corn dextrose. Plasmodia were transferred to the plates in which the fungi were growing and the results of their parasitism on the mycelia are presented in Table 1. The relative terms used in Table 1 and in subsequent tables to describe the degree of parasitism of the myxomycete, based upon the criterion of the rate of digestion of the fungous mycelium, are: "very parasitic," "parasitic" and "not parasitic." "Very parasitic" denotes a case in which the mycelium of the fungus was rapidly and usually completely consumed, as for example, the case of *Badhamia magna* on *Collybia velutipes*. Where "parasitic" is recorded, the mycelium was more slowly and usually not entirely consumed. "Not parasitic" is recorded when the mycelium was not digested, even though the plasmodium may have passed over it.

The incomplete data recorded in Table 1 indicate that a plasmodium cannot always attack the same mycelium when grown on a different medium, for example, *Badhamia rubiginosa* attacked and consumed the mycelium of *Pleurotus ostreatus* growing on corn dextrose agar but avoided the same fungus growing on potato dextrose agar. This would seem to indicate that the nature of the medium plays an important part in rendering the mycelium susceptible to attack by a plasmodium. It should be noted, however, that some species of fungi even on the same favorable medium and exposed to plasmodia capable of vigorous parasitism on other fungi, invariably remain unattacked. For example, *Physarum cinereum* avoided the mycelium of *Fomes pinicola* growing on *Vicia Faba* agar but did attack *Fomes applanatus* hyphae growing on the same agar. Examples further substantiating this principle are brought out in later experiments (Tables 2 and 3).

Table 2 shows species of plasmodia which consumed mycelia grown on corn dextrose agar. Petri plates containing this agar were inoculated with different fungi and when a considerable mycelium had developed, four or five cultures of each fungus were inoculated with a small piece of plasmodium. The plates were then incubated at 22′ C. This work brings out many idiosyncrasies in the ability of the plasmodia to digest different species of fungi. Some slime molds appear to be generally parasitic upon fungous hyphae while others are only selectively so. The length of life of a plasmodium varied under the conditions of the test, but it was rarely longer than that of a healthy plasmodium growing on corn agar without the fungus.

Although the essential process of assimilation of fungous hyphae is the same for all myxomycetes on all kinds of mycelia, there is great variation in the extent to which and the manner in which hyphae are consumed as a plasmodium advances over a fungus colony (Plate 54, figs. 1–10). Sometimes a plasmodium, advancing across a mycelial culture, digests the mycelium with which it comes in contact and leaves a path freed from fungous hyphae, as does *Physarum polycephalum* on *Collybia velutipes*. Sometimes the plasmodium spreads out in all directions, exposing an ever-widening circle of bare agar as it consumes the mycelium; such is true of *Trichia decipiens* Macbr. and *Lindbladia effusa* Rost. on *Daedalea confragosa*. And sometimes, if the mycelium is in a tough appressed layer, the plasmodium removes only the superficial hyphae, as for example, *Lycogala epidendrum* Fr. on *Lenzites betulina*.

TABLE 2.—PARASITISM OF PLASMODIA UPON FUNGI GROWING ON CORN DEXTROSE AGAR.

Fungus / Myxomycete	Badhamia magna	Badhamia rubiginosa	Brefeldia maxima	Fuligo septica	Hemitrichia clavata	Leocarpus fragilis	Lindbladia effusa	Lycogala epidendrum	Physarum cinereum	Physarum flavicomum	Physarum polycephalum	Physarum virescens	Stemonitis fusca	Trichia decipiens
1. Collybia velutipes	VP	—	P	—	P	N	P	P	P	—	VP	P	N	P
2. Daedalea confragosa	VP	P	P	—	N	P	P	P	—	—	VP	N	—	P
3. Fomes applanatus	P	P	—	—	P	—	P	P	P	—	—	P	—	P
4. Fomes fomentarius	P	P	P	P	P	—	P	P	P	—	—	—	—	—
5. Fomes igniarius	VP	—	—	P	—	P	P	P	—	—	—	—	—	P
6. Fomes pinicola	N	—	—	—	—	—	—	—	N	—	—	—	—	—
7. Lentinus lepideus	P	N	N	—	P	P	P	P	—	—	P	N	P	N
8. Lenzites betulina	P	P	P	—	P	P	P	P	—	—	P	P	—	P
9. Pleurotus ostreatus	VP	P	P	P	P	P	P	P	P	P	VP	N	N	P
10. Pleurotus serotinus	VP	N	P	P	P	P	P	P	P	VP	VP	N	—	P
11. Polyporus resinosus	P	N	N	N	N	N	P	N	P	P	P	—	—	P
12. Polystictus nigro-marginatus	—	—	P	P	P	—	P	P	—	—	P	P	—	P
13. Polystictus versicolor	VP	—	—	—	—	—	—	—	—	—	VP	—	—	—
14. Polystictus sp.	P	N	—	—	—	—	P	P	—	—	P	—	—	P
15. Poria sp.	N	N	P	P	—	N	N	P	—	—	P	P	—	P
16. Trametes pini	P	N	P	P	P	N	P	P	—	—	P	P	—	P

N—not parasitic, P—parasitic, VP—very parasitic.

The parasitism of plasmodia on fungi growing on rolled oat agar was tried by the senior author with the following results: *Leocarpus fragilis* slowly consumed hyphae of *Monilia* (*Neurospora*) *crassa* and of a *Penicillium* sp. *Physarum polycephalum* rapidly digested the mycelium of *Alternaria* sp., *Aspergillus* sp., *Cyathus stercoreus*,

TABLE 3.—PARASITISM OF PLASMODIA UPON FUNGI GROWING ON DISKS OF OAT AGAR INSERTED IN PETRI PLATES OF PLAIN AGAR.

FUNGUS \ MYXOMYCETE	Arcyria occidentalis	Badhamia rubiginosa	Fuligo septica	Hemitrichia Vesparium	Physarum polycephalum	Physarum tenerum	Trichia persimilis	Trichia scabra	T	BFU	BH	BI	BJ	BX	HH	C14	C16	C30
1. Armillaria mellea	N	—	P	P	VP	VP	P	P	P	VP	P	—	P	P	VP	P	P	VP
2. Bulliardella sp.	N	—	—	—	VP	VP	—	—	—	—	—	—	N	—	—	—	—	—
3. Collybia velutipes	N	N	N	VP	VP	VP	—	N	N	VP	P	N	N	P	N	N	N	N
4. Coprinus micaceus	VP	N	P	P	VP	VP	N	VP	N	N	VP	N	N	P	P	N	N	N
5. Crucibulum vulgare	N	N	N	N	VP	VP	—	N	N	VP	—	N	N	N	N	N	N	N
6. Daldinia concentrica	N	—	N	P	VP	—	—	—	—	—	—	—	VP	—	—	P	VP	—
7. " occidentalis	VP	—	VP	P	VP	—	—	—	—	—	—	—	VP	P	VP	VP	N	—
8. " simulans	N	—	P	P	VP	—	—	—	P	—	—	—	—	VP	P	P	VP	—
9. " vernicosa	—	—	VP	—	P	—	—	VP	P	—	—	—	—	P	VP	—	VP	—
10. Fomes applanatus	N	—	N	N	VP	P	—	N	P	VP	—	—	N	N	N	N	N	—
11. " fomentarius	N	—	P	N	VP	P	—	N	N	VP	N	—	N	N	N	N	N	—
12. " igniarius	P	N	P	P	VP	VP	—	N	N	VP	N	N	—	P	P	P	N	—
13. Ganoderma oregonense	N	N	N	N	P	N	—	N	N	N	N	N	N	N	N	N	N	N
14. Hypoxylon coccineum	N	—	VP	VP	P	—	—	—	N	—	—	—	N	N	P	P	P	—
15. Lentinus lepideus	N	—	N	N	VP	VP	—	—	N	N	—	—	N	N	N	N	N	N
16. Lenzites saepiaria	N	—	P	N	VP	VP	P	N	N	VP	VP	N	VP	N	N	VP	N	—
17. Mycorrhizal fungus	—	—	—	—	VP	P	—	—	P	—	—	—	—	—	—	—	—	—
18. Mytilidion sp.	P	—	—	N	VP	VP	—	—	—	P	—	—	—	N	N	—	—	—
19. Panus stipticus	VP	N	P	P	VP	VP	—	N	N	—	—	N	N	P	P	P	N	—
20. Pholiota adiposa	N	P	P	N	VP	VP	—	N	N	VP	—	N	N	N	N	N	P	—
21. Pleurotus ostreatus	N	—	N	N	VP	VP	—	N	N	VP	N	—	N	N	N	—	N	—
22. Polyporus betulinus	P	—	VP	P	VP	VP	N	N	N	VP	—	N	N	N	P	P	N	—
23. Polystictus pargamenus	N	N	N	N	VP	VP	N	N	N	VP	N	N	N	N	N	N	N	—
24. " versicolor	N	N	P	N	VP	VP	—	N	P	VP	N	—	N	N	P	N	N	N
25. Schizophyllum commune	N	N	N	N	VP	VP	N	N	—	VP	P	N	N	N	N	N	N	N
26. Trametes pini	N	—	N	N	VP	VP	—	N	P	VP	N	N	N	N	N	—	N	N

N—not parasitic, P—parasitic, VP—very parasitic.

Exidia glandulosa, *Merulius americanus*, *Monilia* (*Neurospora*) *crassa*, *Nidularia pulvinata*, *Penicillium* sp., *Tremella mesenterica*, and *Tremella* sp.; but did not digest the hyphae of *Beauveria globulifera*, *Guepinia spathularia*, and *Mucor* sp. *Physarum viride* plasmodia rapidly digested the mycelium of *Chaetomidium fimeti;* and more slowly digested hyphae of *Aspergillus* sp., *Beauveria bassiana*, *Monilia* (*Neurospora*) *crassa*, and a species of *Penicillium*.

A wide range of species of fungous mycelia capable of being digested by various plasmodia was tested by the disk insertion method (Plate 54, fig. 1), and the results are tabulated in Table 3. Occasionally cultures exhibited a puzzling variation in the digestion of the mycelium on oat agar disks and to obviate this, duplicate cultures were prepared. Indeed, in some instances to give added certainty, if the duplicate cultures showed any discrepancy, three to four additional cultures were made. In such doubtful cases, it is the average behavior of three to five plasmodia that is recorded in Table 3. The variation, however, was generally a question of the rate of digestion of the mycelium by the plasmodium. For instance, *Arcyria occidentalis* slowly consumed the mycelium of *Fomes igniarius* in two trials while in a third trial the plasmodium completely digested the mycelium, but in Table 3 this plasmodium is listed merely as "parasitic" instead of "very parasitic." Likewise, out of five trials of *Physarum tenerum* on *Fomes fomentarius*, three gave evidence of parasitism while the other two did not, yet the plasmodium is listed as "parasitic." As previously stated, the variation was commonly one of the degree of parasitism with but one or two exceptions. One exception occurred in the case of *Fuligo septica* digesting *Lentinus lepideus*, in which the plasmodium died on the mycelium in two cultures, avoided the mycelium in a third, but completely consumed it in a fourth.

The fungus listed as "mycorrhizal fungus" (see Table 3), is a subculture of *Mycelium radicis atrovirens* isolated in Sweden by E. Melin and obtained through the courtesy of Mr. A. B. Hatch. This non-sporulating fungus produces a dark submerged mycelium and a lighter aerial mycelium which was consumed by *Physarum polycephalum*, *Physarum tenerum*, and slightly by plasmodium T.

The behavior of the plasmodia in digesting fungous mycelia seems unquestionably to vary with the medium upon which the fungi are growing. The plasmodia of *Physarum tenerum* and *Physarum polycephalum* more rapidly consumed the mycelium of *Schizophyllum commune* from disks of malt extract agar than from disks of rolled oat agar. Another, more striking example, of the effect of the medium upon the ability of a plasmodium to digest a given mycelium was demonstrated by *Hypoxylon coccineum*. This fungus is very slowly digested on oat agar by the plasmodium of *Physarum polycephalum*, while in repeated trials the same fungus growing on bean pod decoction agar is rapidly consumed by the same myxomycete. It is of interest to note that on the first medium a dark green pigment is produced by the fungus while on the second it is absent, but whether the presence or absence of this pigment is in any way connected with susceptibility to parasitism remains a question. Similarly, a yellow undetermined species (Plasmodium T) digested the white aerial hyphae of *Hypoxylon coccineum* from a disk of bean pod agar but not from a disk of oat agar.

In summarizing the parasitism of plasmodia and the factors influencing it, Tables 1, 2, and 3 demonstrate that the same plasmodium may flourish on the mycelium of one fungus and not attempt to attack that of another species. Plasmodia of the same genus but of different species of slime mold may show as great a variation in parasitism as those of two different genera. In general, it may be said that some Myxomycetes are restricted in choice of host while others seem to be generally mycophagous.

SUMMARY

Laboratory and field observations have disclosed the mycophagous habit of several plasmodia of Myxomycetes other than the fifteen species that the authors (5) recently reported digesting Hymenomycetes, which furthers the hypothesis that plasmodia digest the mycelia of a wide variety of fungi responsible for the decay of wood and debris. The Myxomycetes found to consume fungous hyphae under the conditions of the tests were: 1, *Arcyria occidentalis*, 2, *Badhamia magna*, 3, *B. rubiginosa*, 4, *B. utricularis*, 5, *Brefeldia maxima*, 6, *Fuligo septica*, 7, *Hemitrichia clavata*, 8, *H. Vesparium*, 9, *Leocarpus fragilis*, 10, *Lindbladia effusa*, 11, *Lycogala epidendrum*, 12, *Physarum cinereum*, 13, *P. flavicomum*, 14, *P. polycephalum*, 15, *P. tenerum*, 16, *P. virescens*, 17, *P. viride*, 18, *Stemonitis fusca*, 19, *Trichia decipiens*, 20, *T. persimilis*, and 21, *T. scabra*.

Two methods for testing the parasitic habit were used: one, in which the plasmodia were allowed to attack the fungi on the same nutrient medium upon which the latter were growing, and a second, in which the plasmodia were transferred to petri plates of plain agar, each having an inserted disk of nutrient agar upon which the fungus was growing (Plate 54, figs. 1–2). The mycelia of forty-nine, chiefly wood-inhabiting fungi were tested and were found to be consumed in varying degrees by plasmodia.

Before closing, the junior author wishes to express her thanks to

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EXPLANATION OF PLATE 54

Fig. 1. Plasmodium of *Physarum polycephalum* leaving the transferred piece of agar and moving toward the mycelium of *Trametes pini* growing on an oat agar disk which has been inserted in a petri plate of plain agar. Six hours later the fungous hyphae had been completely consumed. × ½.

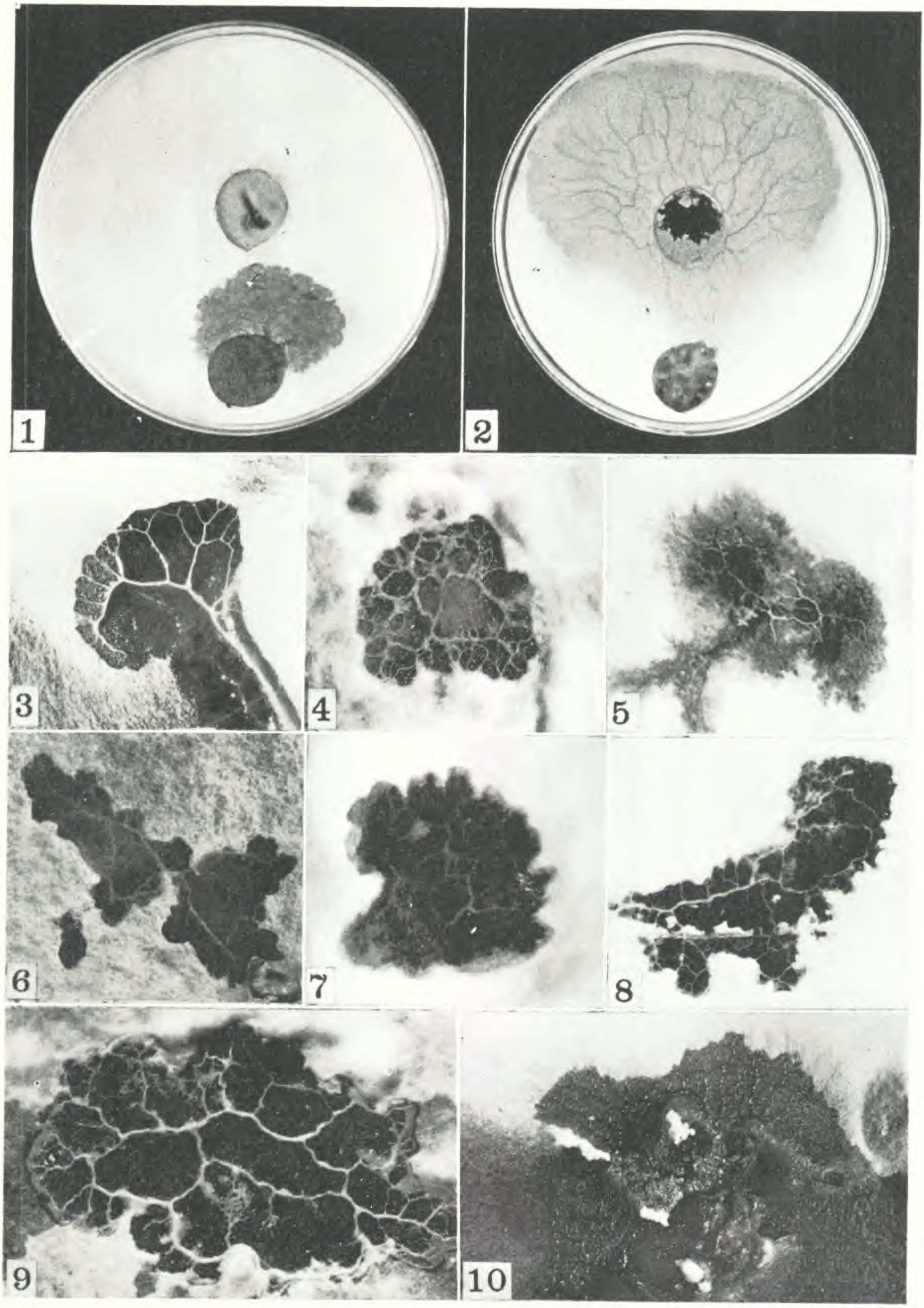
Fig. 2. Plasmodium of *P. polycephalum* consuming the white hyphae of *Fomes igniarius* from an oat agar disk and leaving some of the older dark-colored hyphae.

Fig. 3. Plasmodium of *Physarum virescens* digesting the hyphae of *Fomes applanatus* on corn dextrose agar.

Fig. 4. Plasmodium of *Badhamia rubiginosa* removing the mycelium of *Daedalea confragosa* from corn dextrose agar.

Fig. 5. Plasmodium of *Fuligo septica* dissolving *Fomes fomentarius* hyphae on corn dextrose agar.

Fig. 6. Plasmodium of *Brefeldia maxima* attacking hyphae of *Collybia velutipes.*



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